# June 2016 ISSUE | Rob Knight

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**THE INTERVIEW**

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So here we are in our 2016 podcast version of Functional Medicine Update and really I think we're out of the blocks with a bang, so to speak. We're very fortunate to have, in this edition, Dr. Rob Knight. I think that name probably goes without me giving a lot of description because he's in the news, he's on the marquee, and his work is really at the primacy of where the frontier of this whole field of functional health is going. But just for those of you who might not fully know of Rob's background, let me just quickly give you some of the high points.

He's presently a professor at the University of California, San Diego, and he's the co-founder of the American Gut Project, which has received a tremendous amount of attention. He was educated in New Zealand, and his lab's research presently is involved with the development and computational techniques that are related to characterization of the microbes of humans, animals, and in the interrelationship with the environment. He has a background in biochemistry from a university in New Zealand and his PhD at Princeton, and I found his PhD focus to be really interesting. Only at Princeton, probably, could you have such a wonderful title: The Origin and Evolution of the Genetic Code.[1] He completed his PhD in 2001.

Until 2014 he was a professor at the University of Colorado in Boulder, and now has moved over to UCSD. Having read his recent book, which is titled Follow Your Gut: The Enormous Impact of the Tiny Microbes, I can say that's the starting point for any of you that are wanting to get into more understanding about implications and concepts related to health and disease associated with the microbiome.[2] This is the expert, this is the man, this is one of the people that are leading the charge in really explicating this very complicated story about these thousands of species that reside either as symbionts, commensals, or parasites within the human gut microbiome.

Rob, it's wonderful to have you as our leader of this edition of Functional Medicine Update. Thank you so much.

RK: Well, thank you so much, Jeff, for that overly kind introduction. It's truly an honor to be part of this. Thanks again for the invitation to connect with this community. I think being able to exploit the microbiome for functional medicine is something that is just emerging, but something that holds so much potential and I'm really glad that your listeners are excited about the topic.

JB: Rob, a question that I commonly ask individuals who are leaders in the field is what led you into this area where you're now investing so much of your time, energy, emotion, and your psyche into this field? What drew you into it? It's a very interesting path from biochemistry ultimately into the microbiome.

Early Research Using RNA Molecules to Study Environmental Conditions

RK: Yes, well, you mentioned my PhD thesis on the origin and evolution of the genetic code, and what I was doing there was looking at RNA molecules from the basis of life that might have led to the origination of life in what's called an RNA world billions of years ago before the evolution of DNA and proteins. And so it was really basic studies of RNA that led me to look at how RNA is put together, how it changes in composition and how the sequences change. And one RNA that there was a whole lot of in the sequence databases that I was using was the ribosomal RNA that makes up the ribosome, the factory in our cells that makes proteins. I was curious about why people were gathering so many of these sequences, and actually Norm Pace, who is a National Academy member, a recipient of one of the MacArthur Genius Awards and so forth, was a professor at Boulder at the time I was there, and he was one of people who had collected a lot of these sequences and put them in the database. So I went to his office and asked him why and started coming to his lab meetings. And the reason why is that they weren't so much interested in the structure of that molecule, but in using it as a tool to readout where organisms fall on the tree of life, and place and categorize biological knowledge. So I realized that we could take that tool a step further, where if you were looking at the whole community based on its RNA, instead of using a single RNA molecule as the tool to find out about where the organisms are phylogenetically (in other words, in terms of where they are on the tree of life), what you could do is you could use the whole community of organisms as a tool to find out about the environmental conditions where that community was, whether you're talking about the oceans or the soil or even our own bodies. And so that's what led me to getting interested in this, in a very indirect path.

Now, more directly the events surrounding the birth of my own child a little over four years ago got me thinking about how we could really apply these tools much more directly to health, and so in large part that's what prompted my move from Boulder to UC San Diego, where I'm now in the Departments of Pediatrics and Science and Engineering.

Pioneering a Cross-Disciplinary Collaborative Approach to Research

JB: That in itself is a title that is really a 21st century academic title, isn't it? Because you're crossing disciplines and that would be almost considered breaking the trust or the guild maybe 20 years ago, to have just a person covering that swath of different disciplines.

RK: That's absolutely right. A lot of people think that pediatrics and computer science and engineering is

one department rather than two. Often I joke right now that we're working on a robot that goes "Mom!" Yes, there's a lot of stuff in the works.

JB: I know that Craig Venter and his group have been very interested in charting phylogeny from the oceans and the environment. Is there any kind of interrelationship between what you're doing and the Venter group?

RK: Yes, absolutely. As you know, Craig is really a pioneer in metagenomics, both with the global ocean survey, the expedition of the Sorcerer II mapping oceans around the planet in terms of their microbiology, as well as the Human Microbiome Project with the JCVI. The J. Craig Venter Institute played a very early and leading role. So I worked with a large consortium including the JCVI in the Human Microbiome Project, which was this huge, 172 million dollar project funded by NIH that really provided the overall map and framework for understanding the human microbiome. One thing that's really nice about the density of research institutes here in La Jolla is the JCVI San Diego site is only a couple of miles from my lab, so you can literally just walk there from my lab. We have done it on occasion, and we frequently have people from the JCVI at our lab meetings and that kind of thing, and there are a number of collaborative projects. Not only are we building on techniques that Craig pioneered, but on top of that there's a lot very interesting opportunities for collaboration, both in the environmental space and in the health space.

JB: One of the other interesting people in this field that we've had the privilege of speaking to on Functional Medicine Update is Eric Schadt, and I'm sure you know him and his work. I wonder, has there been any collaboration with what is going on with the Schadt group in translational genomics?

RK: Yes, there have been some interactions there, too. In fact, Jose Clemente, who was a very talented post-doc out of my lab was hired by Eric at Mount Sinai a couple of years ago to add a microbiome dimension to their research programs there, and they have been very successfully working together in that regard. One thing that has been very exciting is seeing how systems have been starting to evolve to embrace the microbiome, and in a lot of ways that makes a lot of sense. Your own metaphor that food is really something that speaks to our genes and it does so in a language of color, and when it speaks to our genes our genes do different things. I always think it's important to remember that although we have a lot of human genes—the human genome has about 20,000 human genes depending upon what exactly you count—that's only a tiny fraction of the genes associated with our bodies. And given that the size of the microbial gene catalog is two to twenty million genes, 99 percent of the genes that you have aren't even in your human genome. It's always important to remember that your food speaks to all those genes as well.

JB: That's a powerful concept. That's a great segue, then. Could you let us know, for those not familiar, you're one of the founders and directors of the American Gut Project. Tell us a little bit about the project, if you would.

The American Gut Project and Citizen Science

RK: Well, the American Gut Project really built on the success of projects like the Human Microbiome Project and other traditionally governmental foundation-funded projects, where what you do is you come up with a design for a study, you write a grant to support the study, you battle for a couple of years to get

that grant funded, then you go through your institutional review board, it takes a year to get approval, you then start to recruit people, and then maybe five years later you have enough data, and maybe five years after that you can write up and publish a paper about the results. And while that's very important and you need those kinds of cohorts to really draw valid scientific conclusions, it leaves a lot of people out in the cold. You know, a lot of your listeners are probably thinking, "Hey, wait, what about my microbiome? How can I get involved in this? Because, you know, I poop, too. Why can't I find out what's in that poop?" And the answer is that the traditional model is not very participatory. So what American Gut is is an effort to bring these technologies that we developed for the Human Microbiome Project and other projects to everybody and make it possible for you to find out what's in your own microbiome in the context of citizen science and open science, which is really a radically new way of conducting scientific research. Effectively what we do is we make it possible for anyone who is interested to basically sign informed consent, participate in the research project, send us their sample, and then we'll tell them how their microbiome looks compared to a huge number of other people. At this point we've had over 8000 people sign up for it. We've released the data—de-identified, obviously, so you can't tell whose sample goes with which, although you can see which is your sample. We've released thousands of samples so that you can see how your data look like in comparison to other people. What's really exciting about this is it builds on the map that the Human Microbiome Project provides us, to tell us where in that space of possible microbiome configurations different groups of people are. So, for example, we're right in the process of looking at some of the healthiest people at UC San Diego, so the student athletes, for example, the people in the healthy aging cohort. And then some of the sickest people, so people in the cardiac ward, people in the oncology ward, people in the IBD clinic, so that we can get a full understanding of where the good places and bad places are on that map, and things that you have control over can move you into a good place or move you into a bad place, so that we can give people guidance about what you should do to optimize your microbiome for health over your lifetime.

The Future of Microbiome Treatments

JB: That is unbelievably exciting, both from a scientific question-and-answer perspective, but also from a methodological perspective. I think that you are pioneering on several levels, there. And by the way, for those that have not followed your publications, you know in the academic world the measure of productivity of researcher is through their publication record and yours is just stellar, both in the quality of the journals you're publishing in and the number of publications you've amassed that are really, I think, of high scientific impact over the last, really, not that many years. One of those—of the many—is a very interesting paper that I really enjoyed in which the title was "Why Microbiome Treatments Could Payoff Soon."[3] That was in Nature, which is obviously a very high profile journal, in 2015. Could you tell us a little bit about what led you into both that title and that article, because I think it says it all. It's a very interesting and provocative article.

RK: Yes, absolutely, and thanks for your kind comments on the publication record. One thing I'll note is that in science, although there's this myth of the lone scientist working away, actually what matters most is teamwork and having great collaborators and great students working with you on these things, and so a lot of what you're seeing is much more being part of a number of really great teams than something that is considered individual accomplishment. I think teamwork is an incredible part of this. In that particular article, what I think is so exciting about the microbiome is both its capacity to diagnose and its capacity for change. It's important to remember that microbiome therapies aren't just something in the future; they are happening right now. For example, there are many people walking around alive now who would

be dead had they not received fecal microbiota transplants for Clostridium difficile-associated disease, or C diff as it is commonly called. But that's a pretty extreme intervention, and in terms of being able to find out what the microbiome means for us in terms of our capacity to respond to drugs, our capacity to respond to diet, and so forth, and if you are doing a diagnostic that can change your behavior or the behavior of your prescribing physician, you can potentially use that information a lot faster than you can use information about a therapeutic, which has to go through a much more rigorous FDA approval process than a diagnostic. And so given that we now know that the microbiome is linked not just to things like obesity, but also to things like our ability to metabolize drugs, ranging from Cyclophosphamide to even acetaminophen, so everything from cancer drugs to pain killers. This has tremendously exciting potential to exploit that knowledge about the microbiome to figure out the best treatment plan.

Now one thing that wasn't even on the radar when I wrote that article is the possibility that the microbiome can tell you what specific you should eat, and two very talented Israelis, Eran Segal and Eran Elinav, had a great paper that came out in Cell right at the end of last year, where what they did is they hooked up continuous glucose monitors to a cohort of 800 people and fed them standardized diets so that they could tell what was the effect of each dietary item for each person on their blood sugar.[4] And what was amazing about that was in terms of blood sugar control, for some people it is actually worse to eat a bowl of white rice than it is to eat a bowl of ice cream. You know, that's the sort of thing where you might really want to know which category you were in, right? Should I forsake the ice cream and focus on the rice, or should I do exactly the opposite? Being able to extend that to the attraction between all kinds of other components of our diet and all kinds of other component systems of our health, that's exactly the kind of thing that we're seeing techniques today that are ideally suited to.

JB: Thank you for bringing up that paper. I think that for those of us that follow Cell on a regular basis—or just follow the field—that was one of those ah-ha papers. When it was published we immediately did a little blogosphere broadcast to our user group about it because I thought it was one of those paradigm-shifting studies, both in terms of the methodology but also in terms of how they were looking, in a systems biology way, at personal differences (individual differences) as we move towards a more precision-based form of healthcare. I think that your work is a linchpin in really adding a huge part to this story. You mentioned drugs. One of them that we know is being used with greater and greater frequency that is influenced significantly by the microbiome is metformin in the management of blood sugar in type 2 diabetics. I think that this cuts across the full spectrum—everything from prevention to tertiary treatment as it pertains to what you are learning.

RK: That's right, and metformin is a fascinating case because it really illustrates the difficulty of getting at causality in these microbiome studies. You might have been following—published in Nature—where a Chinese group essentially reported one set of microbes associated with type 2 diabetes and then a Scandinavian group reported a completely separate set of biomarkers, and a lot of what they were picking up was actually the result of metformin treatment, which was different in the disease between those two populations, and so this is really why we need to do prospective longitudinal studies with a lot of people to figure out what's cause and what's effect in the microbiome, as well as using various preclinical models.[5] If you've been following this stuff, you're probably aware of the work of Jeff Gordon at Washington University and his colleagues, including some of the work my lab participated in where it's possible to transmit phenotypes like obesity or malnutrition from one mouse to another by transferring the microbes, and even more excitingly, transferring from individual people into mice by transferring the microbes.[6] There's a lot of work in the field at the moment basically aimed at asking what other

diseases can we do those kinds of transfers from, where it's almost like you can infect a mouse with obesity. Really, based on the human microbiome, could you infect a mouse with Alzheimer's or with Parkinson's, for example, by transferring the microbes from someone who was sick with those diseases? We don't know the answer to that yet, but we will very shortly.

Examining the Mechanisms of Action Within the Microbiome

JB: So obviously this is right at the frontier of such a dramatic change that if Metchnikoff was alive today he would probably be saying, "I told you that this was important!" It's really, really unbelievably interesting. It bears a question, which I'm not sure if this is a fair question to ask but I'm going to ask it anyway. There are multiple putative mechanisms of action as to how these microbes in the microbiome could influence systemic health. I would say that there are two, maybe, general—and maybe I'm being too limited, but two general ways that I would think about this from a mechanistic perspective. One is that the microbes that represent the gut microbiome have differing cell wall chemistries and they have different lipopolysaccharides, and different types of marker compounds on their surface that interact with different receptors, maybe of the G-protein coupled receptor family or other receptors on the gut epithelia, than then signal through those interactions, or the release of things like LPS of different types through different receptors like the toll-like receptors. I guess I'm going back to Metchnikoff, here, with innate immunity when I talk about that. That's one possible mechanism. Another mechanism is that the gut bacteria—and I know you've explored, by the way, both of these, so I know I'm not asking this question without your knowledge being greater than.

The second is that the microbes have differing physiologies and therefore they produce secondary metabolites that are different, and so you get the effect of their metabolism not directly through receptor binding of their cell wall constituents but through their release of different waste products or secondary metabolic byproducts that then are absorbed and have influence systemically through their influence as signaling substances or metabolic substances on cell-specific activity within the host. Are either or those mechanisms or both of those mechanisms operative, or what's your thought right now?

RK: Yes, absolutely. So we know a lot more about mice than we do in humans in this respect, especially because we can dissect the mice afterwards and really find out what's going on, which is a little bit more difficult to get approval to do with humans, right? And there's also a third mechanism, which is direct neural signaling between the gut and the brain, so for example through the vagus nerve, which can affect a whole range of physiological responses. So in mouse models, it's certain that both of those mechanisms are operating as well as the third one I mentioned. So for example, in terms of secondary metabolites, there's a very nice story from Stan Hazen's lab on TMAO (Trimethylamine N-oxide), where essentially microbes produce TMAO from choline, which is a product of digesting meat and eggs and cheese and so forth, and then that gets processed into TMAO, which can then cause cardiovascular disease if you have particular kinds of bacteria, but not if you lack those bacteria.[7] And then there's another very nice story related to autism with maternal immune activation leading to pups that have an altered microbial community with a lot of the chemical called 4EPS, where that chemical, if you deliver it by itself, can induce a lot of symptoms similar to autism in that mouse model—and remember this is only to the extent…you know, you can't really call it autism, but a lot of the symptoms in the mouse are reminiscent of symptoms in humans.[8] So we know the metabolite mechanism definitely operates. We know for sure the immune mechanism operates. I don't really need to go into detail for this audience, I think. I think a lot of people are familiar with leaky gut and the role of gut barrier dysfunction in causing systemic

inflammation, and that leads to all kinds of downstream influences on diabetes and a range of other conditions with an autoimmune component. And then, intensive gut-brain connections, there has been some very interesting work looking at Lactobacillus probiotics and their effects on anxiety, where basically you can make a timid mouse braver by giving it the right probiotic, but it only works if the vagus nerve is intact, so this nerve that carries signals directly from the gut to the brain.[9] If you stunt that nerve, you don't see any fate of the probiotic anymore. So there is a lot of fascinating work demonstrating that these pathways exist, and what we don't know right now is which of those pathways are really important in human disease and which of those pathways are not important. We also don't yet know which are the right control levers, so which pathways you want to target primarily in order to modify human disease and whether you want to target the bacteria directly, or you want to target the diet, or you want to target a modifiable lifestyle factor, or you want introduce drugs and target the host, or maybe the interaction between some of those variables, and that's all work in progress at the moment.

JB: Wow, exciting, exciting stuff. So one of the many papers that you've authored and had published recently is in Cell Metabolism around microbiome metabolites in health and disease.[10] The reason I'm bringing that back to discussion for a half a second is there are a number of clinicians who use different types of urinary metabolite analyses to do a surrogate analysis of microbial activity in the gut. So this would be like urinary organic acids and certain members of that family. Is there some support, based on what you're finding, for that type if surrogate analysis?

Surrogate Analysis of Microbiome Metabolites

RK: Yes, I think we're going to see a much richer use of metabolomics, not just from urine but from plasma and perhaps directly from stool as well, which is something that we're working on with Peter Dorrestein. He's another member of the leadership team of the Center for Microbiome Innovation that I run here at UC San Diego. We've also been reaching out to Joe Wang, who's a faculty member and director of the Center for Wearable Sensors here at UC San Diego. One thing that's a really exciting prospect is being able to figure out which of the right metabolites you would want to track in the blood on a continuous basis, where basically you can get readouts every minute and deliver them to the subject's smartphone, so you can get them and then upload them to the cloud for analysis. So you can really get into the situation where you have a very rich datastream coming in from continuous monitoring of metabolites. This is analogous to what's being done for glucose already. One thing I'm hoping is to have a more detailed conversation with you later on to try to figure out which of the metabolites we should be most focused on. At this point I'm starting to get the impression, though, that maybe nobody knows, and a lot of what we're going to have to do is basic discovery science to figure out which metabolites and which body products are the best proxy for what's going on, either with our physiology or with our microbiome or with the interactions between them.

JB: Fantastic. As I again just look at kind of a Rorschach evaluation—just a quick snapshot—of your publication record recently: Journal of Neurosciences in 2014, "The Microbiome and the Nervous System"; in 2015, "The Microbiome Influence on the Endocrine System"; Trends in Endocrinology and Metabolism in 2015, "Microbiome and Obesity"; and then that leads us up into these kind of threshold papers that you've authored with your collaborators on things like "Prebiotics and the Microbiome," and "The Microbiome and Diet," and how quickly does the microbiome change—if at all—with dietary change, and can you use probiotics and/or probiotics to actually modulate or modify the human microbiome?[11],[12],[13],[14],[15] I'm asking a big question here, but is there kind of a sound bite

summary that can help directionally point us as to what you're finding in this extraordinary area?

Current Types of Microbiome Interventions: Probiotics, Prebiotics, Symbiotics

RK: Yes, sure. Just in case your listeners don't know this already, the three types of intervention that there is a lot of interest in at the moment are probiotics, where you add some good bacteria directly as live bacteria; there's prebiotics, where you feed them some fertilizer that will help the right bacteria grow; and then there's symbiotics, which is basically putting in bacteria and the right fertilizer. So for example, breast milk is a great example of a symbiotic, because in addition to delivering all the beneficial oligosaccharides and other compounds in the breast milk, the breast milk itself helps promote the growth of the right bacteria, and the breast milk also delivers a lot of bacteria that are beneficial to the baby. So to summarize a large and complex field, basically what we're finding is that all of these strategies, as well as many others like phage therapies, immunotherapies, and so forth—all of these things can alter the microbiome. And there's a lot of research right now showing that you can have a statistically significant effect on the microbiome with those interventions, as well as all kinds of other interventions, like altering exercise, altering sleep patterns, altering diet, obviously, which has a huge effect, especially with respect to the plant component of the diet and all of these different things. Most of the science so far has focused on: you have a control group, you have a group that you put through one of these interventions, and do you see a statistically significant difference? What research hasn't really focused on yet is what is the relative effect size of those differences, and which of those differences promote a healthy versus a disease state? That's what we're really trying to put together with American Gut and with other projects that we can feed into the same framework with the laboratory methods and the data analysis, where what we want to be able to do is to define the regions of the map that are healthy versus not so healthy, and then ask, for all of these interventions, which interventions are saving you and helping you stay in a healthy region versus an unhealthy region? Was that at about the right level of detail you were interested in or were you looking for something more specific?

JB: No, I think that's extraordinarily helpful, because I believe that as with any new exciting area, people get very interested in it, and because obviously most of us don't have even superficially as much knowledge in this topic as an expert such as yourself, sometimes there's an over-reading or an over-interpretation or there is a quick-to-act without really understanding there's a lot more complication below the surface. I'm just trying to put some context to the excitement that people have clinically about this as to what level of knowledge we still have yet to go before we've got the size of the playing field all described.

RK: Right, absolutely. And one thing that's a big issue that probiotics in the United States are regulated as food supplements. As a result, if you are able to demonstrate a clinical endpoint it actually harms rather than helps your product because then you're re-regulated as a drug and you have to go through a very cumbersome approval process. So most of the clinical trials that have been done demonstrating probiotics that actually work have been done in Europe or Australia or other countries outside the United States. But one huge issue at the moment is that the public enthusiasm for probiotics is greatly outstripping the actual evidence. Although there are some probiotics that have been very carefully validated in clinical trials—so things like post-antibiotic diarrhea or for irritable bowel syndrome or inflammatory bowel disease. Those are typically different probiotics for different conditions. The state at the moment is kind of along the lines of someone telling you, "Well, you know, I felt sick, so I took a chemical and then I felt better. So obviously what I think is that chemicals are great and everyone should take a lot of chemicals every day

to promote their health." You probably have a lot of follow-up questions about that, right? Like, what were you sick with? And, what chemical did you take and has it actually been clinically validated as a drug or did you just get it from some guy off the street? And you should really have equivalent questions about probiotics, because you can think of the genome as being an insanely complex and precise chemical, but it consists of millions of different substances you put together in a very precise arrangement. It's not just a chemical itself, but a factory for producing thousands to millions of other chemicals. And when you think of it that way, it just makes a lot of sense that different probiotics would do different things. And remember that they're living organisms as well, so it's almost analogous to the promotion of a plant-based diet, right? There's a lot of evidence coming in from different groups, including your own, that a diverse, plant-based diet is really beneficial for health, but at the same time you don't recommend to people that they should eat any plant that comes their way, right? You want to be a bit smart about which plants, for example a tomato rather than a deadly nightshade.

Use of Fecal Microbiome Analysis in Clinical Decision-Making

JB: Thank you. I think that was a very eloquent summary of a whole variety of deep-level information that helps guide our thinking. A lot of clinicians who are getting fecal microbiome analyses done are looking at the Firmicutes and Bacteroidetes families of bacteria and making clinical decisions about the health or the status of that individual's microbiome. Could you just give us a quick comment about those two as markers?

RK: Yes, so remember that Firmicutes and Bacteroidetes are bacterial phyla and to give you an idea of phyla in the animal world, the arthropod phylum includes everything from wasps to lobsters, right? The mollusk phylum includes everything from like a clam to an octopus. So you're talking about very broad groupings of organisms, where within those groupings they do different things. What we're seeing, and this has been seen in many different projects including the Human Microbiome Project, is that there is tremendous variation in most phyla and the relative abundance, even within healthy people. So although if you have a large population you can get statistically significant differences between groups based on those phylum-level classifications, the variation within each of those groups is much larger than the variation between groups, so that's not necessarily going to be a healthy diagnostic. Now at the same time, if you take the data and you feed it into a machine learning classifier, what you can do frequently is you can classify someone as, say, lean or obese with 90 percent accuracy based on their microbial profile, using a machine learning algorithm called Random Forest, but you can't do it by eye. And 90 percent accuracy for obesity, although it's an impressive technical trick that we can do for the microbiome, because remember you can only do that task with 58 percent accuracy from the human genome versus 90 percent accuracy based on the microbiome. You'd have to have a computer algorithm to do it. You can't do it by eye and you can't do it just from one number. What is going to develop is as we get better information in a consistent way about different diseases, what you'll probably move away from is looking at the raw taxonomic output, and what you'll move towards is having an indicator that tells you directly what is your progression towards a particular disease and what is your risk of developing it in the future. So, for example, we're starting to get engaged in a project where we're looking at thousands of stool samples that we collected 15 years ago from people with complete medical records, where we're going to be able to predict what are the bad things that happened to them based on the stool sample from before the disease developed. In many ways this is really helpful, right? Because the last thing, as a clinician, you want is for someone to come into your office with a list of a thousand species, or even worse, a list of a million genes, and, you know, you've got 15 minutes with them and what do you get to

tell them about that list? I guess at the moment the best thing you can do is refer them to a psychiatrist for being crazy enough to think that they could get out of that information in its present form. But I mean it's just very difficult to grapple with. So in terms of what you can get out of those phyla right now, you can probably tell them a fair bit about their diet, which is always an interesting party trick, I guess. And if you have enough data—like if you have enough people—you can tell systematic differences that, taken as an aggregate, reveal a lot of things about their lifestyle and about their health. But you can't do it just from one number, and you can't do it just from those phylum-level representations, and ideally you want to look at how the person changes over time.

So Larry Smarr, who is the director of CAL ITT, which is one of the major research institutes here at UC San Diego, and he was the founding director of NCSA, in charge of the team that developed Mosaic, which intended to navigate and founded the whole graphical web browser idea and so forth. He's now been very interested in his own gut microbial ecology. He'd been staring at those kinds of pictures for years, of how much different phyla you have in the gut, and it was only when we got samples over time and used some of the techniques that we used in American Gut at the whole profile level that we finally understood what was going on, which is we can see very clearly in his data that he switches between two different microbial states, and one of those states is correlated with feeling really bad with IBD symptoms, and the other state is correlated with feeling fine, gaining weight rather than losing it, and having very few IBD symptoms. So moving away from those kinds of taxonomic readouts towards a readout that is more directly connected to function is going to be really important. Now, the basis for that is going to be the kind of sequencing you're doing right now, where you get the taxonomy, but you don't want to look at the taxonomic data any more than you want to look at 1s and 0s that make up a picture on your smartphone, right? You'd much rather see it as a picture than try to decipher it from the binary.

JB: That was an extraordinarily helpful explanation for the clinicians listening. Thank you very much. How much of this changing composition of the microbiome is an effect of changing metabolism and how much is the cause of a changing metabolism? So you get this interesting diet, don't you, as it relates to these approaches that you're questioning?

RK: Absolutely. Well, with causality you can get at when you can do interventions and that's something that I think as a clinician you have a remarkable to do on a therapeutic basis because you're recommending that you patient does something, then if they do it and you see a change you can reasonably attribute it to the intervention, especially if it's repeatable and you can see them switching back and forth as they go on and off the intervention. One thing that is developing from our forensics project is that we do a fair amount of forensics work funded by the National Institutes of Justice, is we can find out a remarkable amount about someone from their microbiome and from their metabolites. One thing that's very interesting in terms of the things that are shaping up for the future is you might be able to tell whether your patients are compliant with what you're prescribing, either in terms of taking drugs, or for example, in wildlife applications there has been a lot of work doing things like trying to figure out what does a seal or a penguin eat? It's very difficult to strap a camera on them—is it going to stay on underwater or not, or have enough drag to slow them down? So what you can actually do is you can use the DNA of the organisms they ate to readout their diet directly, and so we may be able to this with humans as well. Of course, this is only going to work if they're eating real food, because if you're eating mostly highly refined foods there's not going to be any DNA left in it, but that in itself may be really useful for figuring out are your patients eating real food and thus has the full spectrum of secondary metabolites that we probably have evolved with and eat as humans, or are they very eating highly

processed and refined diets where there's not enough DNA left to detect?

Studying the Microbiome and the Health of Infants

JB: That's fascinating. One last question. I want to come full circle, back to where you started. You gave us a teaser early on saying that one of the reasons that you left Colorado and joined the faculty at UCSD, and I'm sure there are many reasons but one of them that you teased us with was that you became a father. You and your wife had your child and this whole concept of the importance of the microbiome and the health of infants. And I noticed that in 2016 you have a very, very interesting paper in Nature Medicine, another high-tier scientific publication, on vaginal transfer and influence on neonates. Tell us a little bit about that, maybe in close, as we come full circle.[16]

RK: Sure, absolutely. So when we had our child towards the end of 2011, being in Boulder and exposed to that kind of environment, we had a very detailed birth plan. We were hoping things were going to go naturally, so we were planning for vaginal delivery, minimal use of drugs, breastfeeding, all of those kinds of things. We had worked all this out with our care team. And, as anyone who has kids knows, things don't necessarily go according to plan once you have kids on the scene. That was true with us as well, so we wound up having to have an unplanned C-section. Then, for various reasons, breastfeeding didn't work out either despite going through the whole parade of medical professionals, so everyone from lactation consultants to psychiatrists to figure out why. So that really got me digging into the evidence for a lot of the ideas that we have about what's healthy and unhealthy in the birth and neonatal context, especially with respect to things like breastfeeding, for example. If breastfeeding doesn't work out, should you bottle feed with a specific formula, should you use other milk, does it matter if that other milk is pasteurized, which is going to kill the bacteria and potentially denature some of the proteins? What are the cost benefit trade-offs and so on? If you're thinking about finite resources, should you put your resources primarily into your kids' diet or should you be looking at other things, like for example hiring additional care so that the mother can get some sleep and be better rested, which is one of those things that there's excellent evidence for, especially when there are other issues. And so what was fascinating to me was how little we know about a lot of those processes. In some sense, it's being able to bring microbiome science right to the beginning of life and being able to help address a lot of those questions, but also being able to intercept people early on, as young children, and being able to take what we're learning about the microbiome and how it intersects with diet and so forth and using that in a way that can promote health over someone's lifetime. That was a very compelling motivation to move to a medical school and to really be in a position where I could work closely with clinicians on clinical problems and figure out how we can use the best technology to make progress in those areas.

JB: I just want to close by acknowledging something that I think is quite remarkable beyond the scope and depth of what you shared with us, which is really quite remarkable, what you're accomplishing as a scientist. But I want to talk about methodology. What is a scientist? What is translational research? What are high impact projects? And what is leadership in a field all about? My feeling, having had the privilege of speaking to and knowing many pace-leading scientists over the years is that what you're doing and how you're doing it fulfills all those criteria of a true scientist of distinction. You're obviously crossing barriers, you're crossing various levels of specialization, you're venturing into new areas. There's always risk in being first to prospect in a new area for fear of all sorts of things. And yet you're being guided by not only the keenness of your mind but by a spirit of inquiry that is coupled together with this ability to make sense out of this work and make it translatable into improving the health and function of

people. It's quite remarkable. I feel actually very privileged to know that there are people like you coming up that are doing this kind of work and approaching it in the way that you're doing it. It's the kind of work that really makes a difference in our society.

Rob, thanks so much. I think both as an admirer of your work and also as a person who is really very much a student of the history of science I think that you are really doing some extraordinary work with you and your team and this collaborative model you've talked about—this distributive model, this translational model—this is the science of the 21st century and I really appreciate both the way both you are doing it and how you communicate it.

RK: Thanks so much for those kind comments. I don't really know how to response to that other than to say that it certainly means a lot to hear those kinds of things from a leader in the field and in this community such as yourself. I guess the way I think about it is a lot of the things that we're doing in terms of trying to explore completely new areas are very risky and—coming from an evolutionary biology background—I always worry a little bit about whether, when you're taking strategies that are increasing the variability of your outcome, you know, if it works then it can be really spectacular, but of course there's the other tail of the distribution that you could equally probably have been at, and keeping that in mind when evaluating people who are interested in joining the lab and taking those risks, it's always important to try to ensure that they have good career outcomes themselves. I think actually so much of it is about being open to new ideas, being open to teamwork, and being open to learning from other communities that you might not have considered before, and being able to apply techniques from other fields. And one thing I'm really excited about is a lot of these issues were grappling with in the microbiome, you've already faced in the natural products community and in the nutrition community. One thing that inspires me is that there is still a tremendous amount that we don't understand about nutrition, but at the same time if you look at the chronic diseases of a century ago, like scurvy, pellagra, beri beri, and so forth, those diseases that sickened millions and incapacitated or even killed very large numbers of people are just gone now, right? You won't meet anyone who's met anyone who's met anyone who has goiter probably, which used to be a very prevalent condition. The idea that we could do the same sort of thing in the microbiome, where even before we fully understand something and the full complexity of the community we may be able to come up with simple, safe, and effective interventions that we can apply either at the precision level to individuals or at the whole population level and have that kind of impact. That's what I think is really inspiring and what I think is really worth doing as vigorously as we can.

JB: Well, thank you. I think that's a wonderful way, I think, to bring this discussion to a close. We wish you, obviously, great success and we'll be following you and your group's work very closely. Thanks so much for spending so much time with us. This message will resonate around the world I'm sure and be transferred from person to person. It's really a lot of news to use.

RK: Thanks again, Jeff. Thank you for what you're doing. You're such an eloquent spokesman for the role of diet in promoting and maintaining our health and figuring out how to add the microbiome into that I think is spectacular, so thanks again for your interest.

JB: Thank you. Best to you. Bye.

RK: Thanks. You, too.

## Bibliography

[1] Knight, Robin Douglas. *The Origin and Evolution of the Genetic Code: Statistical and Experimental Investigations*. Diss. Princeton University, 2001. Web. 27 May 2016. Retrieved from: http://www.ece.iit.edu/~biitcomm/research/Genetic{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36}20Code/The{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36}20Origin{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36}20and{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36}20Evolution{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36}20of{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36}20the{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36}20Genetic{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36}20Code{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36}20Statistical{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36}20and{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36}20Experimental{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36}20Investigations{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36}20-{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36}202001.pdf

[2] Knight, Rob and Brendan Buhler. *Follow Your Gut: The Enormous Impact of Tiny Microbes* (TED Books). New York: Simon & Schuster/TED, 2015.

[3] Knight R. Why microbiome treatments could pay off soon. Nature. 2015 Feb 26;518(7540):S5.

[4] Zeevi D, Korem T, Zmora N, Israeli D, Rothschild D, et al. Personalized Nutrition by Prediction of Glycemic Responses. Cell. 2015 Nov 19;163(5):1079-94.

[5] Forslund K, Hildebrand F, Nielsen T, Falony G, Le Chatelier E, et al. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. Nature. 2015 Dec 10;528(7581):262-6.

[6] Ussar S, Griffin NW, Bezy O, Fujisaka S, Vienberg S, et al. Interactions between Gut Microbiota, Host Genetics and Diet Modulate the Predisposition to Obesity and Metabolic Syndrome. Cell Metab. 2015 Sep 1;223):516-30.

[7] Zhu W, Gregory JC, Org E, Buffa JA, Gupta N, et al. Gut Microbial Metabolite TMAO Enhances Platelet Hyperreactivity and Thrombosis Risk. Cell. 2016 Mar 24;165(1):111-24.

[8] Hsaio EY, McBride SW, Hsien S, Sharon G, Hyde ER, et al. The microbiota modulates gut physiology and behavioral abnormalities associated with autism. Cell. 2013 Dec 19;155(7):1451-1463.

[9] Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, et al. Ingestion pf Lactobacillus strain regulates emotional behavioral and central GABA receptor expression in a mouse via the vagus nerve. Proc Natl Acad Sci U S A. 2011 Sep 20;108(38):16050-5.

[10] Sharon G, Garg N, Debelius J, Knight R, Dorrestein PC, Mazmanian SK. Specialized metabolites from the microbiome in health and disease. Cell Metab. 2014 Nov 4;20(5):719-30.

[11] Mayer EA, Knight R, Mazmanian SK, Cryan JF, Tillisch K. Gut microbes and the brain: paradigm shift in neuroscience. J Neurosci. 2014 Nov 12;34(46):15490-6.

[12] Neuman H, Debelius JW, Knight R, Koren O. Microbial endocrinology: the interplay between the microbiota and the endocrine system. FEMS Microbiol Rev. 2015 Jul;39(4):509-21.

[13] Rosenbaum M, Knight R, Leibel RL. The gut microbiota in human energy homeostasis and obesity. Trends Endocrinol Metab. 2015 Sep;26(9):493-501.

[14] Eilam O, Zarecki R, Oberhardt M, Ursell LK, Kupiec M, et al. Glycan degradation (GlyDeR) analysis predicts mammalian gut microbiota abundance and host diet-specific adaptations. MBio. 2014 Aug 12;5(4). pii:e01526-14.

[15] Org E, Parks BW, Joo JW, Schwartzman W, Kang EY, et al. Genetic and environmental control of host-gut microbiota interactions. Genome Res. 2015 Oct;25(10):1558-69.

[16] Dominquez-Bello MG, De Jesus-Laboy KM, Shen N, Cox LM, Amir A, et al. Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer. Nat Med. 2016 Mar;22(3):250-3.

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